

High hyperdiploid acute lymphoblastic leukemia in adults shows clonal heterogeneity and chromosomal instability at diagnosis and during the course of the disease

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Dear Editor,

High hyperdiploidy with 51–67 chromosomes (HeH) constitutes a large cytogenetic subset of B cell precursor childhood acute lymphoblastic leukemia (ALL) [1]. It is much less common in adult B cell precursor ALL where it was reported in nearly 10% of patients for whom outcome was improved compared to the other cytogenetic groups,

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but not as favorable as in children [2, 3]. It is rarely found in T cell or mature B cell ALL.

Automated four-color interphase fluorescence in situ hybridization (I-FISH) previously revealed a high level of clonal aneuploidy heterogeneity in HeH ALL at presentation [4]. Numerical chromosome instability (CIN) was supposed to be at the origin of this heterogeneity. To assess the presence of clonal heterogeneity and numerical CIN in adult HeH ALL at diagnosis and during disease course, we focused on a series of ten ALL patients selected according to the presence of HeH by conventional cytogenetics, age, and availability of material for four-color I-FISH investigation using probes specific to chromosomes 4, 6, 10, and 17 (Supplement 1). Probes were chosen based on the frequent gain of these chromosomes in HeH ALL and its prognostic significance [5]. Patients were referred between 1995 and 2009 from the university hospitals of Basel, Zurich, Bern, Lausanne and cantonal/regional hospitals of St-Gallen, Aarau, Mendrisio, Bellinzona, and Genolier Clinic. Two patients were enrolled in the SAKK ALL 33-86/90 [6] and GRAALL 2005 clinical trials, respectively. Ethical approval for this project was obtained in accordance with the guidelines of the local Ethical Review Board.

Thirty-four samples were analyzed (presentation, 7; hematologic remission, 19; relapse, 8); status of heterogeneity and CIN level were determined (Table 1, Supplement 2). Significant aneuploidies were identified based on cutoff values defined according to the Poisson distribution, and combinations of aneuploidies were considered relevant when at least one aneuploidy was determined to be significant. Average CIN was determined for all four chromosomes together and then for each selected chromosome. Approaches used and cutoff levels were reported in detail previously [4, 7].

Table 1 Clones involving chromosomes 4, 6, 10, and 17 at presentation and/or during the course of disease in ten patients with high hyperdiploid acute lymphoblastic leukemia, expressed as percentages

Patients	Clones expressed as %																	
	1 ^a	2 ^a	3 ^a	4 ^a	5	6	7	8	9 ^a									
Analysis	131/05 ^b	88/06 ^b	363/09 ^b	241/05 ^b	1086/07 ^b	601/02 ^c	261/06 ^c	331/06 ^c	1282/07 ^d	1181/08 ^c	29/09 ^d	540/09 ^d	2385/95 ^b	1527/97 ^c	27/98 ^e	158/98 ^c	388/02 ^b	95/03 ^c
Normal	18.4	51.0	9.8	53.8	20.7	44.2	86.4	54.6	87.0	42.7	98.6	99.4	24.2	21.7	74.2	96.8	49.6	77.4
+4	8.6	3.8	—	9.0	13.0	5.3	4.8	14.3	3.5	3.3	—	—	1.2	11.4	—	2.6	—	—
+6	—	2.6	6.2	4	28.3	9.3	—	—	3.7	4.4	—	—	17.2	5.4	4.4	—	—	—
+10	15.8	2.2	8.6	—	—	6.4	2.0	4.6	—	3.4	—	—	15.0	3.0	9.4	—	45.8	19.8
+17	5.4	2.6	7.6	—	—	2.2	—	—	3.5	2.6	—	—	—	—	—	—	—	—
+6, +17	—	—	7.4	—	—	—	—	—	—	2.6	—	—	—	—	—	—	—	—
+6, +10	—	4.0	20.2	—	—	13.9	—	—	—	7.8	—	—	31.2	14.3	9.2	—	1.2	—
+4, +17	4.8	1.2	—	—	—	—	—	—	—	1.1	—	—	—	—	—	—	—	—
+4, +6	—	2.6	—	8.8	34.3	1.7	—	—	—	1.6	—	—	—	3.3	—	—	—	—
+4, +10	16.8	3.6	—	—	—	1.4	5.6	24.3	—	2.8	—	—	—	3.7	—	—	—	—
+10, +17	9.2	1.2	10.8	—	—	—	—	—	—	2.0	—	—	—	—	—	—	1.2	—
+4, +10, +17	14.0	2.4	—	—	—	—	—	—	—	2.1	—	—	—	—	—	—	—	—
+4, +6, +10	—	5.2	—	—	—	2.1	—	—	—	5.4	—	—	—	13.4	—	—	—	—
+6, +10, +17	—	1.8	23.2	—	—	—	—	—	—	6.4	—	—	1.0	—	—	—	—	—
+4, +6, +17	—	1.6	—	—	—	—	—	—	—	1.9	—	—	—	—	—	—	—	—
+4, +6, +10, +17	—	7.0	1.0	—	—	—	—	—	—	6.0	—	—	—	—	—	—	—	—
+4, +4	—	1.2	—	8.6	—	1.5	—	—	—	—	—	—	—	1.8	—	—	—	—
+4, +4, +6	—	—	—	13.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
+4, +4, +6, +10	—	1.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
+6, +6	—	—	—	—	—	—	—	—	—	—	—	—	1.4	2.8	—	—	—	—
+6, +6, +10	—	—	—	—	—	—	—	—	—	—	—	—	3.0	1.6	—	—	—	—
+4, +6, +6, +10	—	—	—	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	—
+4, +6, +10, +10	—	—	—	—	—	—	—	—	—	—	—	—	—	2.5	—	—	—	—
+6, +10, +10	—	—	—	—	—	2.7	—	—	—	—	—	—	—	2.1	—	—	—	—
+6, +10, +17, +17	—	—	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
+4, +10, +17, +17	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Others ^e	5.8	4.6	4.0	2.2	3.7	9.4	1.2	2.3	2.3	3.9	1.4	0.6	5.8	11.5	2.8	0.6	2.2	2.8
Total of abnormal clones	21 (13)	29 (13)	24 (15)	13 (8)	18 (15)	44 (34)	6 (3)	8 (5)	19 (16)	40 (25)	7 (7)	3 (3)	20 (13)	56 (43)	12 (9)	2 (1)	8 (5)	7 (6)

Values in parentheses are numbers of very small abnormal clones (<1%). Clones already present in the previous analysis (<1%) are italicized. Study patients are further detailed in Supplement 1. Four-color I-FISH using centromeric probes specific for chromosomes 4 (p-4n1/4, kindly provided by Prof. Mariano Rocchi, University of Bari, Italy), 6, 10, and 17 (D6Z1, D10Z1, and D17Z1, respectively; American Type Culture Collection, Manassas, VA) was performed as formerly described [4] on 34 bone marrow samples, 5 of which were reported in Talamo et al. [7]. Probes were directly labeled by nick translation with four different fluorochromes (Cy3, Cy3.5, DEAC, and FITC). Automated four-color I-FISH analysis was realized with the scanning system Metafer 4/MetaCyte (MetaSystems, Altlußheim, Germany) using a motorized epifluorescence microscope (Axiolmager Z1; Zeiss, Feldbach, Germany) equipped with a ×40 objective (Zeiss). In each sample, a minimum of 500 interphase nuclei were scored, except in one case (331/06) in which only 350 nuclei were available for classification

^a Patients in part reported by Talamo et al. [7] (12)

^b Presentation

^c Relapse

^d Hematologic (bone marrow) complete remission

^e Cumulated percentage of very small clones (<1%)

High levels of clonal heterogeneity were observed at diagnosis and during disease course, at relapse particularly. Clones detected at presentation generally reappeared at relapse, mostly accompanied by newly generated ones (Supplement 3). Whereas the mean total number of abnormal clones did not clearly differ between diagnostic and relapse samples, the range of their variation did, being much larger at relapse (Table 1). Despite the small number of patients, a significant correlation was observed between number of abnormal clones and CIN, suggesting that the higher the instability, the larger the number of abnormal clones (Fig. 1).

Data let surmise that the dynamic process at the origin of HeH ALL is a complex one leading to coexistence of sub-clones with different combinations of aneuploidy, whose heterogeneity and variation result from a simultaneous chromosome gain mechanism driven by underlying chromosome instability and whose evolution will depend on natural selection and acquisition of additional genetic abnormalities [1, 8].

Given the poor outcome associated with CIN in solid tumors and myelodysplastic syndromes [9, 10], the nature and extent of clonal heterogeneity at diagnosis may be of prognostic significance in HeH ALL. This question would merit to be investigated in a large cohort of HeH ALL patients.

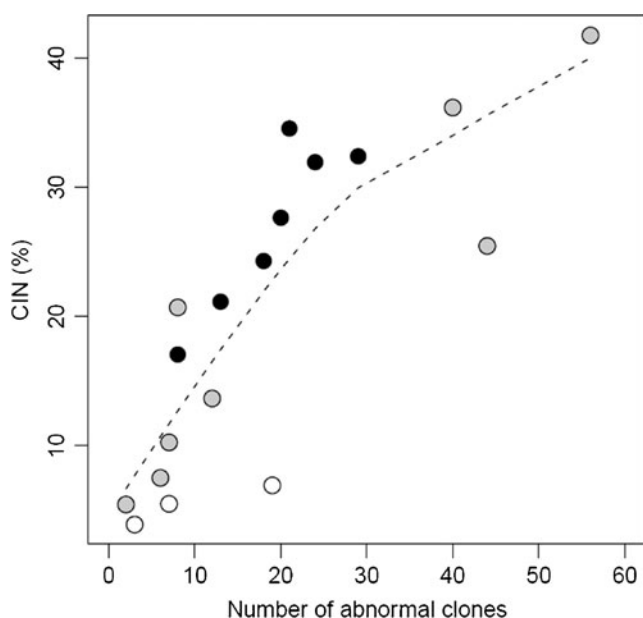


Fig. 1 Number of abnormal clones and CIN values (%) in 18 bone marrow samples from 10 patients with high hyperdiploidy acute lymphoblastic leukemia at disease presentation (*black points*), hematological complete remission (*white points*) and relapse (*gray points*). The correlation between number of abnormal clones and CIN level was measured using the Spearman correlation coefficient. There is an increasing curvilinear trend, as suggested by the broken line (a nonparametric smooth). The Spearman coefficient (0.89) was highly significant ($p < 10^{-6}$).

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